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IN VITRO ANTHELMINTIC ACTIVITY OF *ACMELLA OPPOSITIFOLIA*

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ABSTRACT

Background and Objectives: *Acmella oppositifolia* is an Indian medicinal plant belongs to the family Asteraceae. The present study has been designed to screen the anthelmintic activity of ethanolic extract of *Acmella oppositifolia*. **Methodology:** To evaluate the *in vitro* anthelmintic activity of ethanolic extract of *Acmella oppositifolia* we have selected the adult Indian earthworms, *Pheretima posthuma*. In this study dried extract was taken in 1% w/v carboxymethyl cellulose and prepared in normal saline in three different concentrations (25, 50, 100 mg/ml). The suspension of Albendazole taken in same three concentration considered as standard and normal saline water with 1% CMC was taken as a control. All the worms were placed in petri dish containing 15 ml of sample (drug) solution. Time taken for paralysis (Vermifuge) was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water (50 ^{0}C). Death (Vermicidal) was included when the worms lost their motility followed by white secretions and fading away of their body colour. **Results:** Ethanolic extract of *Acmella oppositifolia* has exhibited significant anthelmintic activity at all the tested concentrations when compared to standard drug since vermifuge and vermicidal effect was highly significant at the higher concentration (100 mg/ml). **Conclusion:** The current investigation leads to conclusion that the *Acmella oppositifolia* have potent anthelmintic activity when compare with the standard albendazole drug. Therefore, we can use this plant as an alternative source of anthelmintic drug and the activities may probably due to the presence of flavonoids, glycosides, saponins, alkaloids and triterpenes.

KEYWORDS: *Acmella oppositifolia*, Anthelmintic activity, Albendazole, Vermifuge,Vermicidal.

INTRODUCTION

A person gets helminthiasis when there is an exposure of body part with worms. These helminths are normally present in the digestive tract but may also found inside the liver and sometimes other organs also.[1] Helminthiasis occurs worldwide, especially in tropical and subtropical countries where poverty is high.[2] Intestinal worms such as *Haemonchus contortus* have been found to have developed resistance to some anthelmintics, namely, levamisole, albendazole, and closantel.[3] Apart from the development of anthelmintic resistance,[4,5] most of the drugs used to treat these worm possess some common side effects such as nausea, vomiting, abdominal pain, and low blood pressure in humans.[6] Again, the issue of anthelmintic resistance in humans has become a matter of concern thus posing a serious threat to the drug manufacturers. Therefore, there is a need to develop other alternatives for the control of helminthiasis so medicinal plants are among the natural products being explored for their anthelmintic potentials.[7,8,9,10,11]

Acmella oppositifolia is belongs to the family Asteraceae. It is native to America and has been introduced to Asia, Africa, the Pacific islands, and Australia. The flowers, leaves and stem are used for oral infections: they reduce toothache and cure canker sores or small mouth ulcers. Very less research studies have been carried out on the anthelminthic activities. This study, therefore, evaluated *Acmella oppositifolia* for its anthelmintic properties.

MATERIALS AND METHODS

Collection and authentication of stem bark

The stem of *Acmella oppositifolia* was collected from the campus of Bharathi College of Pharmacy, Bharathingar. The Plant has authenticated by the Dr. Mahesh HM, Assistant Professor and Head, Department of Botany, Bharathi College, Bharathinagara, Maddur Tq, Mandya Dist. Karnataka. The sample was dried in shade at room temperature for two weeks to obtain uniform dry weight. The dried stems were ground into a coarse powder and stored at 25°C in an air sealed container until the analysis was started.

Preparation of ethanol extract of *Acmella oppositifolia* stem

The powdered plant material (40 g) of *Acmella*

oppositifolia bark was weighed and subjected to maceration technique using the ethanol solvent system (400 ml). It is shaken occasionally for 48 hours days at room temperature. The contents of the flask were filtered through Whatman filter paper number 1 the filtrate was concentrated using a steam evaporation. The extract was kept at 4°C in the refrigerator until needed. Its percentage yield was calculated to be 9.7%.

Phytochemical screening

The phytochemical screening of ethanol extract of *Acmella oppositifolia* was carried out to detect the presence or otherwise of secondary metabolites such as tannins, alkaloids, and flavonoids, following standard procedures.

Selection of test organisms[12]

In this experiment we have used Indian adult earthworms (*Pheretima posthuma*) because of its anatomical and physiological similarity with the intestinal round-worm parasite of human beings. All the earthworms were collected from the wet soil of agricultural land. For the removal of all fecal matter from worms they were washed with normal saline. The earthworms of 7-10 cm in length and 0.3-0.5 cm in width were used for all the experimental protocol.

Evaluation of anthelmintic activity[13,14]

The assay for anthelmintic activity was carried out. Six earthworms were aseptically transferred into properly labelled petri dishes six in each group. Dried extract was suspended in 1% w/v Carboxymethylcellulose, prepared in normal saline water in three different conc. (25, 50, 100 mg/ml). Albendazole suspension of same conc. was taken as standard and normal saline water with 1% CMC was taken as a control. Worms were placed in petridish containing 15 ml of sample (drug) solution.

Group –I. Control: Vehicle (1% CMC in normal saline)
Group - II. Standard: Albendazole (25, 50, 100 mg/ml)
Group - III. Test: Ethanol extract (25, 50, 100 mg/ml)

Statistical Analysis

All experiments were carried out in triplicate. Results were presented as mean ± standard deviation (SD). All data were statistically analysed by one-way ANOVA using Graphpad Prism 2019 program to determine whether there were any statistically significant differences between the means of two or more independent groups using P- value ≤ 0.05**.**

RESULTS AND DISCUSSION

Phytochemical screening

The extracts were obtained from each of the solvents were subjected to thequalitative chemical tests to detect the major active constituents. The phytochemical analysis revealed that the flavonoids, tannins, triterpenes, saponins and glycosides are present in the ethanolic extract whereas the alkaloids, phenols and sterols were absent.

Table 1: Qualitative chemical investigations of extracts of *Acmella oppositifolia.*

Sl. No	Phytochemical tests	Ethanolic extract of stem
1	Alkaloids	-
2	Glycosides	+
3	Tannins	+
4	Phenols	-
5	Flavonoids	+
6	Saponins	+
7	Sterols	-
8	Triterpenes	+

"+" = present, "–" = absent

Table 2: Anthelmintic activity of ethanolic extract of *Acmella oppositifolia*.

Group/treatment	Concentration	Time (In Minutes)	
		Paralysis	Death
Control (1% CMC)	-	-	-
Ethanolic extract	25mg/ml	35.01±0.164 *	28.63±0.05 *
	50mg/ml	20.42±0.093 **	26.43±1.04 **
	100mg/ml	16.69±0.048 **	24.093±0.326**
Albendazole (Standard drug)	25mg/ml	30.17±0.971	31.43±0.25
	50mg/ml	18.14±0.410	34.38±0.59
	100mg/ml	13.106±0.562	26.483±0.128

All values are expressed as mean ± standard deviation (SD)Significant level P<0.05 *, P<0.01 **
Moderately significant [*] highly significant [**]

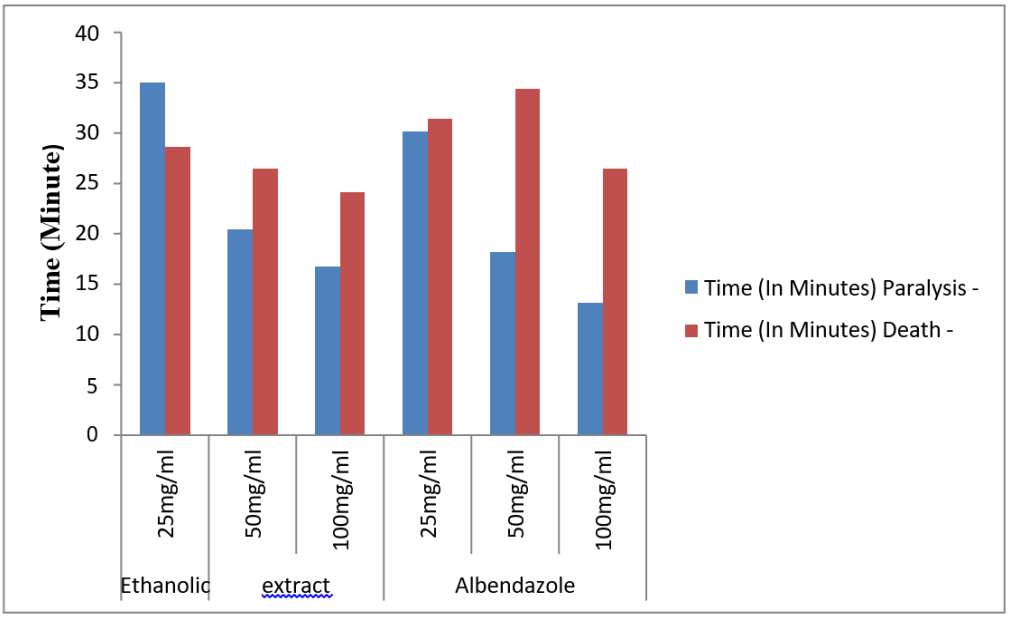

Figure 1: Anthelmintic activity of *Acmella oppositifolia* on *Peretima posthuma*.

Death time of worms at 25, 50, & 100 mg of ethanolic extract

The paralysis time of worms were observed in both the treated and standard group at different concentration. The worms treated with 25 mg of ethanolic extract of *Acmella oppositifolia* was found to be moderately significant ($p<0.05$) when compared to worms treated with standard drug albendazole.

The worms treated with 50 mg of ethanolic extract of *Acmella oppositifolia* was found to be highly significant ($p<0.01$) when compared to worms treated with standard drug albendazole. The worms treated with 100mg of ethanolic extract of *Acmella oppositifolia* was found to be highly significant ($p<0.01$) when compared to worms treated with standard drug albendazole.

DISCUSSION

Medicinal plants are undoubtedly rich sources of biologically active compounds vital to human health. If used properly, medicinal plants are a solution toa wide range of diseases *Acmella oppositifolia* is one such medicinal plant being used in folk medicine for treating various disorders.

For past 22 years, an attempt to establish the pharmacological value of *Acmella oppositifolia* through rigorous scientific investigations has been taken by researchers all over the world. Despite the differences in term of cultural, geographical, location, climate, different parts of *Acmella oppositifolia* have been medicinally used to treat various ailments and have been scientifically proven.

Antioxidant activity improvement in endothelial function, vascular function, and insulin sensitivity, as well as attenuation of platelet reactivity and reduction in blood pressure. Moreover proper scientific screening of potential bio actives of these plants followed by chemical investigations is necessary to make these herbal remedies more viable. In this context, the present study was undertaken to evaluate the antioxidant of *Acmella oppositifolia*.

Acmella oppositifolia by produce in dose dependent manner of *in vitro* anti anthelmintic activity, the 25mg takes 38.15 minutes for paralysis and 70 minutes for death of worms, 50 mg/ml takes 25.08 minutes for paralysis and 53 minutes for death of worms and 100mg/ml takes 19 minutes for paralysis and 38 minutes for death of worms. In this project we used albendazole as standard in 25 mg/ml, 50 mg/ml and 100 mg/ml dose manner respectively and it takes 12 minutes for paralysis and 32 minutes for death of worms, 9 minutes for paralysis and 28 minutes for death of worms and 5.30 minutes for paralysis and 15 minutes for death of worms in dose of 25, 50 and 100 mg/ml as shown in table 2.

The present study was undertaken to evaluate the Anti anthelmintic activity of ethanolic extracts of *Acmella oppositifolia* using experimental model as Indian earthworm *(Peretima posthuma).*

Preliminary phytochemical screening of crude extract of the stem of *Acmella oppositifolia* revealed the presence of flavonoids, alkaloids, triterpenes, saponins, glycosides and tannins. Table Based the results the ethanolic stem extract of *Acmella oppositifolia by* produce in dose dependent manner of in-vitro anti anthelmintic activity, the 25mg takes 38.15 minutes for paralysis and 70 minutes for death of worms, 50 mg/ml takes 25.08 minutes for paralysis and 53 minutes for death of worms and 100mg/ml takes 19 minutes for paralysis and 38 minutes for death of worms. In this project we used albendazole as standard in 25 mg/ml, 50 mg/ml and 100 mg/ml dose manner respectively and it takes 12 minutes

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CONCLUSION

The results of the present study clearly indicated that the crude ethanol extract of *Acmella oppositifolia* has showed significant anthelmintic activity against Indian earthworm *Peretima posthuma*. The plant possesses significant anthelmintic activity at 100 mg/ml concentration measured by time taken for paralyze/death of the earthworms.

The current investigation leads to conclusion that the *Acmella oppositifolia* have potent anthelmintic activity when compare with the standard albendazole drug. Hence, we can use this herb as alternate source of anthelmintic drugs and also can isolate active chemical constituent for anthelmintic activity from extract.

From the above results, it is concluded that all the ethanolic extracts of *Acmella oppositifolia* have potent anthelmintic activity when compared with the conventionally used drug and is potent to standard anthelmintic drug. Further studies using in vitro models are required to carry out and establish the effectiveness and pharmacological rationale for the use of *Acmella oppositifolia* as an anthelmintic drug. The drug may be further explored for its phytochemical profile to identify the active constituent responsible for anthelmintic activity.

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